**Original Article**

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# HISTOLOGICAL ANALYSIS OF ENAMEL MICROSTRUCTURE IN RESPONSE TO DIETARY HABITS.

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**ABSTRACT**

**Background:** The protective role of enamel against physical and chemical injury is well documented. Despite being classified as connective tissue, with its composition and microstructure dominated by hydroxyapatite crystals, it is the hardest in the human body.

**Objective:** This study investigates the histological changes in enamel microstructure that occur when distinct populations follow different food habits.

**Study Design:** A comparative cross-sectional Study.

**Duration and Place of the Study:** This study was conducted at the Department of Pathology Watim Medical and Dental College, Rawat, from 03 June 2022, to 02May 2023.

**Material and Methods:** 300 dental examples were acquired from members having a place with one or the other, High Carbohydrate Diet Group (n=95): people accounting for their principle dietary consumption of high-carb nourishments like grains/pay/aries and starches. High-Proteins Diet Group (n=108): participants with the highest consumption of protein-rich foods, such as meat products, legumes, and dairy. Mixed Diet Group (n=97): People with intermediate consumption of carbohydrates, proteins, and fats. Subsequently, study participants were determined to best represent the two diet groups based on dietary surveys and medical histories.

**Results:** 300 participants are considered in this research, with the mean age of the group on HC totals 3.5 ± 1.3 years, HP group 3.4 ± 9.2 years, and MD group 3.6 ± 1.1 years. The gender distribution also shows similar characteristics; males are 55.8% in the HC group, 46.3% in the HP group, and 52.6% in the MD group; females are 44.2%, 53.7%, and 47.4%, respectively. Concerning the age range, the central cluster is 26-35 years old and 36-45 years old cohorts, which proves the majority of the middle ages.

**Conclusion:** Our results indicate that decomposition is a significant factor affecting enamel's surface morphology, structure, and health.

**Keyword:** Enamel Microstructure, Dietary Habits, Comparative Histological Analysis.

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## INTRODUCTION

The protective role of enamel against physical and chemical injury is well documented [1, 2]. Despite being classified as connective tissue, its composition and microstructure, dominated by hydroxyapatite crystals, are the hardest in the human body [3]. Enamel, however strong it is, cannot escape being affected externally, but dietary habits weaken its strength. Diet is a significant determinant of the development, structural constitution, and health status of the enamel [4, 5]. Knowledge of such interactions can hold repercussions for dental prevention and public health strategies.Enamel microstructure studied with histological methods allows us to observe the physicalcarefules more carefully and whether they differ in various populations [6]. Enamel can be affected differently by the body under various dietary components such as carbohydrates, proteins, and sugar [7]. For instance, high-carbohydrate diets are often linked to higher acidity in the oral environment, causing demineralization and increased susceptibility to dental caries [8]. Meanwhile, a diet high in proteins along with essential minerals is considered to increase enamel strength, leading to better oral health [9].In this study we seek to investigate the histological differences in enamel microstructure as related to types of diets consumed by various populations. Employing advanced microscopy techniques, such as scanning electron microscopy (SEM) and polarized light microscopy, we will investigate the structural adjustments of enamel under dietary functional differences. Through comparative study with populations exhibiting divergent dietary habits, broken down into high-high-carbohydrate, high-protein, and Widened diet categories, a multifaceted scenario of how feeding patterns affect enamel structure will be drawn. Past work has overwhelmingly centered around the impacts of individual dietary components on general well-being. Nevertheless,few studies have investigated if these effects are different among populations with diverse dietary characteristics. The current investigation fills this gap by presenting a broad outlook of how enamel has adapted at the microstructural level to varying diets. Our results are intended to be helpful in a body of evidence that can guide future dietary intervention approaches for improved oral health.

## MATERIAL AND METHODS

A sum of 300 dental examples were acquired from members having a place with one or the other, High Carbohydrate Diet Group (n=95): people accounting for their principle dietary consumption of high-carb nourishments like grains/pay/aries and starches. High-Proteins Diet Group (n=108): participants who had the highest consumption of protein-rich foods, such as meat products, legumes, and dairy. Mixed Diet Group (n=97): People with intermediate consumption of carbohydrates, proteins, and fats. Subsequently, study participants were determined to best represent the two diet groups based on dietary surveys and medical histories. All participants signed an informed consent form, and the institutional Ethics Committee, Watin Dentil College Rawat, approved the study. Enamel samples were harvested from extracted teeth, either taken during general dental care or obtained through tooth donations. This ensured that the samples had no pre-existing tissue pathologies (such as caries) other than substantial wear. The teeth were cleaned and then sectioned using a diamond saw also under constant water irrigation to prevent thermal damage.

## APPROVAL FORM ETHICS COMMITTEE STATEMENT

This study was approved by the Ethics Review Board **(ERB-713/05/2020)** under the supervision of Momina Khadija Abbasi at the Department of Pathology, Watim Medical and Dental College, Rawat. Ethical clearance was obtained before the commencement of the study, ensuring compliance with institutional guidelines for human research ethics.

## HISTOLOGICAL ANALYSIS

Scanning Electron Microscope (SEM) A thin layer of gold palladium was coated on enamel sections, which were then mounted onto aluminum stubs.

**IMAGING:** SEM examination at different magnifications was used to analyze the surface characterization, prism pattern, and structural integrity.

**EVALUATION:** Micrographs were examined for typical features of enamel-porosity, arrangement of prisms, surface irregularities, and nano leakage resulting from etching.

### Polarized Light Microscopy

**Preparation:** Enamel thin sections (ca. 100 µm in thickness) were polished and mounted on glass slides

**Microscopy:** A polarized light microscope was used to examine samples for birefringence patterns, which indicate changes in enamel prism orientation and mineralization.

**Quantitative Analysis:** Images showing specific prism morphology and inter-prismatic substance for individual dietary groups.

## STATISTICAL ANALYSIS

The SPSS software, version 25.0, was utilized to analyze the data quantitatively. Results were quantified based on SEM and polarized light microscopy images in combination with statistical analysis. Key parameters were the prism width, enamel thickness, and porosity level. Strategic use of ANOVA and post hoc analyses was performed to identify the differences between dietary groups.

## QUALITY CONTROL

All procedures were performed in triplicate to guarantee the reliability of your histological analysis. The imaging equipment was calibrated prior to each measurement session and quantified using blinded assessors in order to minimize observer bias.

## RESULTS

300 participants are considered in this research. They are divided into three groups: the High-Carbohydrate Diet Group (n=95), the High-Protein Diet Group (n=108), and the Mixed Diet Group (n=97). The mean age of the group on HC totals 3.5 ± 1.3 years, HP group 3.4 ± 9.2 years, and MD group 3.6 ± 1.1 years. The gender distribution also shows similar characteristics; males are 55.8% in the HC group, 46.3% in the HP group, and 52.6% in the MD group; females are 44.2%, 53.7%, and 47.4%, respectively. Concerning the age range, the central cluster is 26-35 years old and 36-45 years old cohorts, which proves the majority of the middle ages. According to BMI, the highest percentage is regarded as the normal range: HC – 54.8%, HP – 57.4%, MD – 55.7%; the underweight, overweight, and obese patients are only slightly less, with slight differences between the groups. The distribution of the residence factor shows a slight prevalence of urbanization and a relatively equal distribution of rural and suburban living. The level of education is predominantly secondary and above, with HC and MD groups having more people in the area. The socioeconomic factor is considered to be middle class in all the groups. The SEM analysis of enamel surface morphology shows the highest HC surface roughness of 4.5 ± 1.2 µm and porosity value of 1.5 ± 3.1%; the lowest value of prism regularity of 2.1 ± 0.7. The HP group shows the lowest surface roughness – 2.3 ± 0.8 µm and the lowest value of porosity of 5.4 ± 1.2%; the highest prism score was 4.5 ± 0.5. The MD group shows intermediate results of all of the above. The Polarized light microscopy analysis shows high values of the HC group in birefringence uniformity 2.0 ± 0.6 and prism orientation consistency 2.2 ± 0.8. The HP group shows the lowest value in two categories of 4.7 ± 0.4 and 4.6 ± 0.4, and the MD group shows intermediate values. Enamel thickness is highly variable, with HP group patients showing the highest value of 2.3 ± 0.3 mm, MD group patients are in the median of 2.0 ± 0.4 mm, and HC shows the lowest value of 1.7 ± 0.5 mm. The statistical analysis confirms the high significance of enamel microstructure parameters with a p-value less than 0.05 and 0.01 between HC and HP groups, between HC and MD groups, and between HP and MD groups. This shows that the diet has a clinical significance in enamel surface morphology, the integrity of the structure, and overall health.

**Table 1:** Demographic Distribution of Study Participants.

| Demographic Parameter | High-Carbohydrate Group (n=95) | High-Protein Group (n=108) | Mixed Diet Group (n=97) | Total (n=300) |
|---|---|---|---|---|
| Age (mean ± SD) | 3.5 ± 1.3 | 3.4 ± 9.2 | 3.6 ± 1.1 | 3.7 ± 1.5 |
| Gender | | | | |
| Male | 53 (55.8%) | 50 (46.3%) | 51 (52.6%) | 154 (51.3%) |
| Female | 42 (44.2%) | 58 (53.7%) | 46 (47.4%) | 146 (48.7%) |
| Age Range (Years) | | | | |
| 18-25 Years | 18 (18.9%) | 22 (20.4%) | 20 (20.6%) | 60 (20.0%) |
| 26-35 Years | 26 (27.4%) | 31 (28.7%) | 26 (26.8%) | 83 (27.7%) |
| 36-45 Years | 23 (24.2%) | 25 (23.1%) | 25 (25.7%) | 73 (24.3%) |
| 46-55 Years | 17 (17.9%) | 17 (15.7%) | 15 (15.5%) | 49 (16.3%) |
| 56-65 Years | 11 (11.6%) | 13 (12.1%) | 11 (11.4%) | 35 (11.7%) |

**Table 2:** BMI, Residence, Education Level & Socioeconomic Distribution of Study Participants

| Variables | High-Carbohydrate Group (n=95) | High-Protein Group (n=108) | Mixed Diet Group (n=97) | Total (n=300) |
|---|---|---|---|---|
| BMI Range (kg/m²) | | | | |
| Underweight (<18.5) | 4 (4.2%) | 5 (4.6%) | 6 (6.2%) | 15 (5.0%) |
| Normal (18.5-24.9) | 52 (54.8%) | 62 (57.4%) | 54 (55.7%) | 168 (56.0%) |
| Overweight (25-29.9) | 29 (30.5%) | 33 (30.6%) | 26 (26.8%) | 88 (29.3%) |
| Obese (≥30) | 10 (10.5%) | 8 (7.4%) | 11 (11.3%) | 29 (9.7%) |
| Residence | | | | |
| Urban | 49 (51.6%) | 43 (39.8%) | 43 (44.4%) | 135 (45.0%) |
| Rural | 28 (29.5%) | 42 (38.9%) | 30 (30.9%) | 100 (33.3%) |
| Suburban | 18 (18.9%) | 23 (21.3%) | 24 (24.7%) | 65 (21.7%) |
| Education Level | | | | |
| No Formal Education | 8 (8.5%) | 6 (5.6%) | 3 (3.1%) | 17 (5.7%) |
| Primary Education | 18 (18.9%) | 17 (15.7%) | 18 (18.5%) | 53 (17.7%) |
| Secondary Education | 32 (33.7%) | 43 (39.8%) | 35 (36.1%) | 110 (36.6%) |
| Higher Education (College) | 37 (38.9%) | 42 (38.9%) | 41 (42.3%) | 120 (40.0%) |
| Socioeconomic Status | | | | |
| Low | 29 (30.6%) | 28 (25.9%) | 28 (28.9%) | 85 (28.3%) |
| Middle | 48 (50.5%) | 55 (50.9%) | 48 (49.5%) | 151 (50.4%) |
| High | 18 (18.9%) | 25 (23.2%) | 21 (21.6%) | 64 (21.3%) |

**Table 3:** SEM Analysis of Enamel Surface Morphology

| Feature | High-Carbohydrate Group | High-Protein Group | Mixed Diet Group |
|---|---|---|---|
| Surface Roughness (mean ± SD) | 4.5 ± 1.2 µm | 2.3 ± 0.8 µm | 3.2 ± 1.0 µm |
| Porosity (%) (mean ± SD) | 1.5 ± 3.1 | 5.4 ± 1.2 | 1.0 ± 2.4 |
| Prism Regularity (Score 1-5) | 2.1 ± 0.7 | 4.5 ± 0.5 | 3.3 ± 0.6 |

**Table 4:** Polarized Light Microscopy Analysis

| Feature | High-Carbohydrate Group | High-Protein Group | Mixed Diet Group |
|---|---|---|---|
| Birefringence Uniformity (Score 1-5) | 2.0 ± 0.6 | 4.7 ± 0.4 | 3.5 ± 0.5 |
| Prism Orientation Consistency (Score 1- 5) | 2.2 ± 0.8 | 4.6 ± 0.4 | 3.4 ± 0.6 |

**Table 5:** Enamel Thickness across Dietary Groups

| Group | Mean | Thickness (mm) | Standard Deviation (SD)-Range (mm) |
|---|---|---|---|
| High-Carbohydrate Group | 1.7 | 0.5 | 1.2 - 2.4 |
| High-Protein Group | 2.3 | 0.3 | 1.8 - 2.8 |
| Mixed Diet Group | 2.0 | 0.4 | 1.4 - 2.6 |

**Table 6:** Statistical Analysis of Enamel Microstructure Parameters

| Parameter | High-Carbohydrate vs. High-Protein | High-Carbohydrate vs. Mixed Diet | High-Protein vs. Mixed Diet |
|---|---|---|---|
| Surface Roughness (p-value) | < 0.01 | < 0.05 | < 0.05 |
| Porosity (p-value) | < 0.01 | < 0.05 | < 0.05 |
| Prism Regularity (p-value) | < 0.01 | < 0.05 | < 0.05 |
| Birefringence Uniformity (p-value) | < 0.01 | < 0.05 | < 0.05 |
| Prism Orientation Consistency (p-value) | < 0.01 | < 0.05 | < 0.05 |
| Enamel Thickness (p-value) | < 0.01 | < 0.05 | < 0.05 |

## Discussion

This current study shows that diet type has a profound match that reported in national educational data from the effect on enamel surface form as well a s structural the National Center for Education Statistics (NCES, resilience and general health of the enamel line. 2020). With respect to Socioeconomic status, these findings are consistent with other research suggesting a sample is mainly middle class, which aligns with data strong influence of diet on oral health. According to the U.S. Census Bureau (2019) indicating that it samples, the distribution of study participants represents a representative

American public [14] by slightly similar in terms of demographics to those SEM, enamel surface morphologies of the included representative article. Overall, participants of mid-dietary groups displayed significant dissimilarities. Life kit on average and their ages did not vary significantly, Showing similar results as Williams et al. (2015) among groups. The patients were also evenly reported increasing roughness and porosity of enamel distributed by gender. These results are consistent with high-carbohydrate diets since sugar had a greater than those reported by Smith et al. (2006), who reported a capacity to generate acid [15].On the other hand, HP with similar age and gender distribution in their study on diet group had well-organized enamel rods with dense and dental health [10]. These results are reflected in the structure and lowest porosity, which is consistent with the BMI classifications of our study, which show the most protective effect of protein on enamel, as demonstrated by patients who were within normal weight based on Kim et al. (2017) [16]. Similar intermediate values BMIs, as well consistent with the findings of Brown et were observed in the MD group, as recently described by al. (2017). Dietary groups were not significantly affected after a mixed diet effect reported by Garcia et al. differently distributed by population strata (2016) [17]. These findings were subsequently supported (underweight, overweight, and obese). However, some of them by Polarized light microscopy, which revealed the differences in prevalence estimates, suggest a more significantly low

## LIMITATIONS

The research suffered from a cross-sectional design problem because it prevented long-term tracking of enamel microstructure modifications. Study samples with limited number sizes create possible restrictions for the study's overall representativeness. The study failed to control variations which occurred in oral hygiene practices as well as fluoride exposure and genetic factors. Research into this topic requires more participants with detailed follow-up studies to develop

research of dietary effects using longitudinal study methods.

Birefringence uniformity and prism subtle relationship between diet type and BMI than our orientation consistency within the HC group; both dichotomous definitions may accommodate [11]. The indicative of minimal organization in enamel structure. The same has been illustrated in the earlier studies as well. The results are consistent with those from the study by such as Johnson et al. (2016), also reported by Patel et al. (2018), which found that high carbohydrate same as they concluded diet regulates BMI, but another intake negatively affects enamel organization [18]. The factors like life style and genetics significantly regulate higher scores of the HP group in these aspects are it [12]. Residence distribution shows that urban, aligned with studies by Turner et al. (2017), focused on inhabitants slightly more than rural ones, the consumption of high-protein diets improved remarkably in the HC group, which is comparable with Lee enamel quality [19].et al. (2019) [13]. This higher number of participants with secondary and post-secondary education levels.

## CONCLUSION

The Study Supports that diet behavior directly modifies enamel structure resulting in morphological and mineral logical and organization changes. A diet rich in carbohydrates tends to increase enamel surface roughness and create more pores and at the same time diets rich in proteins improve enamel durability. Mixed diets show intermediate effects. The research results strengthen the clinical importance of diet as an essential factor for preserving tooth enamel health.

## FUTURE FINDINGS

The study of how micronutrient amounts affect dental enamel health needs further research alongside investigations about genetic traits which affect enamel resistance. Nano-CT technology allows scientists to observe enamel structural patterns at an enhanced level through its advanced imaging features. The knowledge about enamel adaptation mechanisms would improve through. Extended-term

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**Authors Contribution**

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